FORMULATION, EVALUATION OF HERBAL EMUGEL FOR TOPICAL DRUG DELIVERY AND ITS *IN VITRO* SCREENING FOR PRELIMINARY PHYTOCHEMICALS, ANTIMICROBIAL AND ANTI-INFLAMMATORY EFFECTS

Development of Topical Polyherbal Emulgel And Evaluation of its Antimicrobial and Anti-Inflammatory Activities.

SYNOPSIS OF THE THESIS

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DECLARATION

I hereby declare that the dissertation synopsis entitled "**Development of topical polyherbal emulgel and evaluation of its antimicrobial and anti-inflammatory activities**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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1.0 Introduction

The herbal medicines are the oldest form of medicines known to human world and are widely used in the developing countries due to their traditional usage and acceptability. These medicines are prepared from plant sources and are used to treat a number of diseases and health problems all over the world [1]. The entire world is now returning towards the use of herbal medicine system due to their lesser side effects [2]. The drug delivery system is designed to ensure the better entry and reach of the drug to the affected are in the body. Many pharmaceutical companies have patented their own drug delivery systems. Precise dosing and delivery time and metabolic pathway of drug are calculated while designing such systems. Patients prefers those methods which are painless and easy [3]. Topical drug delivery systems are the systems which are used to apply drugs directly to the skin for the treatment of coetaneous disorder. These systems confirm the pharmacological effect of the particular drug formulation to skin surface. The most used topical drug delivery systems include gels, creams and ointments. Among such formulations, topical gel formulation provides one of most suitable drug delivery system due less grease and easily removing ability from the skin surface. The Gel formulations provide better drug application and stability as compared to cream and ointments [4]. Emulgel is one of topical delivery system that provides the properties of gel and emulsion and demonstrates dual release control. The emulgel formulations can be used to deliver hydrophobic drug into skin and provide better stability, loading capacity and controlled drug release. Emulgel offers many dermatological advantages and therefore may serve as better drug delivery system in dermatology [5]. Emulgel which are mixtures of emulsion and gel are one of the novel topical drug delivery system. In this emulgel, an O/W or W/O emulsion from oil and a gel formulation is prepared [6]. The emulgel for dermatological use are thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life and bio-friendly and can serve as better topical drug delivery systems as compared to conventional systems available in market [7]. The usage of emulgels can be expanded in analgesics, anti-inflammatory, anti-fungal, anti-acne drugs and various other cosmetic formulations [8]. The use of gel seems to be more beneficial for both cosmetics and pharmaceutical preparations in comparison to other semisolid formulations. The studies on preparation and characterization of herbal emulgel will

provide better future in delivery of more numbers of topical drugs as compared to other drug delivery systems [9]. The present study has been designed for the formulation and characterization of polyhedral emulgel using plant sources for topical drug delivery and will be evaluated for its antimicrobial and anti-inflammatory studies. These formulations will have the natural drug components which will be safer for use and will have lesser associated side effects.

2.0 Review of literature

2.1 Background

The herbal medicines are the oldest form of medicines known to human world and are widely used in the developing countries due to their traditional use and lesser associated side effects. These medicines are prepared from plant sources and are used to treat a number of diseases and health problems all over the world [1]. The entire world is now returning towards the use of herbal medicine system due to their lesser side effects [2]. The drug delivery system is designed to ensure the better entry and reach of the drug to the affected are in the body. Many pharmaceutical companies have patented their own drug delivery systems. Precise dosing and delivery time and metabolic pathway of drug are calculated while designing such systems. Patients prefer those methods which are painless and cost effective [3]. Topical drug delivery systems are the systems which are used to apply drugs directly to the skin for the treatment of coetaneous disorder. These systems confirm the pharmacological effect of the particular drug formulation to skin surface. The most used topical drug delivery systems include gels, creams and ointments. Among such formulations, topical gel formulation provides one of most suitable drug delivery system due less grease and easily removing ability from the skin surface. The Gel formulations provide better drug application and stability as compared to cream and ointments [4].

2.2. Herbal based medicines

Medicinal plants serve as an integral part of traditional medicine system for many years [10]. Since the ancient times, people are continuously using plants and their products for the prevention and treatment of various infections all over the world. People are using plants and their extracts found in their native places even before the knowledge to existence of microorganisms. Around 80% of world's population are using herbal medicines for their health care [11]. The world health organization has estimated that around 80% of the population of the developing countries depends exclusively on traditional medicine system for their primary health care. It has been estimated that more than half of the people around world still rely entirely on the plants and the phytochemical compounds from these for medicines [12].

Utane and co-workers (2017), studied the effects of various herbal drug formulations for the growth and care of hairs. These workers demonstrated that HS is the product which

not only be used for hair washing but also can be used as a remedy on various problems associated with hairs. The greener preparation of HS was prepared into two sections and all the products demonstrates that the herbal HS is highly suitable for use in daily life for the care of hairs [2].

Heydarpour and co-workers (2018), reviewed the clinical trials undergoing on the effectiveness of medicinal plants and their phytochemical compounds in the treatment of recurrent aphthous ulcers (RAU). These workers searched five databases such as PubMed, Science Direct, Web of Science, Scopus, and Cochrane Library for retrieving data about the clinical trials. These workers indicated that a number of evidences has approved the therapeutic potential of medicinal plants and their products in the management of RAU. The phytochemicals present in these plants have diverse pharmacological and therapeutic benefits for the patients. However, further clinical studies are still required to study the efficacy and safety of plant based products in the treatment of RAU [13].

Kumar and co-workers (2013), has reviewed the medicinal benefits of the divine tree neem (*Azadirachta indica*) which is mainly cultivated in the Indian subcontinent. This plant is widely used by the human population for the treatment of various health issues for many years [12].

Jain and co-workers (2015), have evaluated six plant extracts and 0.2% chlorohexidine for the antibacterial activity against *Streptococcus mutans* clinical isolates recovered from the plaque samples of 45 pediatric patients. All the extracts were prepared in different forms and evaluated for their antimicrobial effects. These workers demonstrated that the crude garlic extract had greater antibacterial activity than chlorhexidine and aqueous extract of amla and organic solvent-based extract of ginger had maximum antibacterial activity. Their study suggests that plant extracts have antimicrobial effects and therefore have great potential for use as an alternative treatment option for the management of dental caries [14].

2.3 Topical drug delivery systems

A number of pharmaceutical preparations are included in topical drug delivery systems such as semisolids, liquid preparation, sprays and solid powders. Among semisolid preparations, gel, creams and ointments are widely used. Gels are a cross-linked polymeric preparation in a liquid medium and exhibit no steady-state flow. The

interaction between polymeric state and the liquid dispersion medium produces an interlacing three dimensional network of particles of dispersed phase. The topical gel formulations may be a suitable topical drug delivery system due to less greasiness and easy removal from the skin. Therefore, gel formulations demonstrate better application properties and stability as compared to other preparations [4]. While designing a drug delivery system, the dosage form, drug metabolism, and route of administration are calculated. The major goal of such systems is to deliver the right dosage of drug to the right place at right time [3]. Topical drug therapies using cream, ointment, gel and lotion are an important part of dermatological therapeutic procedures. Emulgel is novel topical drug delivery system with many benefits [6].

2.4 Emulgel as topical drug delivery system

Emulgel is one of the topical drug delivery system that shares the properties of gel and emulsion and exhibit dual release control system. Recently many antimicrobial, antiviral as well as non-steroidal anti-inflammatory drug formulations are studied for their topical delivery through emulgel formulations [5].

When gel and emulsion are used in combined form, the hydrophobic drugs are delivered easily to systemic circulation through skin. The emulgel have longer shelf life, bio-friendly and transparent appearance [7]. Emulgel is the promising drug delivery system for the delivery of hydrophobic drugs and are commonly used for the delivery of analgesics, anti-inflammatory, anti-fungal, anti-acne and various drug formulations [9].

Sultana and co-workers (2016), used leaf extract of *Lantana camara* for the preparation of an emulgel and investigated their ability in healing excision wounds in diabetic rats. These workers evaluated ethanolic extract of dried leaves of *Lantana camara* for presence of various phytochemicals. They prepared emulgel formulations using different types of gelling agents and impact of these on drug release from emulgel was studied. Their results suggest that herbal emulgel prepared from ethanolic extracts of *Lantana camara* possess significant improvement in excision wound contraction and therefore, can serve as potential candidate for wound healing in diabetic rats [15].

Anwar and co-workers (2014), used capsaicinoid as an active component in the preparation of emulgel and gel formulations. Capsaicinoid is mainly used in topical treatment to get relief from the pain of peripheral neuropathy such as post-herpetic neuralgia or as a cream muscle and joint pain in arthritis. These workers measured

penetration power of capsaicinoid extracted from chili fructus and evaluated the physical stability and other properties of the emulgel and gel prepared from the capsaicinoid. Their results showed that the emulgel was physically more stable with high penetration power than gel [16].

Ahsan and co-workers (2020), done preliminary phytochemical screening and *in vitro* anti-inflammatory assays of the methanol extract of roots of *Saussurea lappa* (SLRE). These workers prepared herbal emulgels and in situ gels using various concentrations of SLRE and assessed these for organoleptic and physical properties. These workers demonstrated that SLRE had 42.8% inhibition in heat-induced hemolysis on erythrocyte membrane model in comparison to aspirin which served as positive control. All the formulations were found stable after one-month stability check at 40°C (emulgels) and 25°C (in situ gel) respectively. Their results indicated that in situ gel prepared from herbal extract had significant wound healing effects and thus suggest the preparation gel formulation using herbal extracts with better potential for wound healing [17].

Ashara and co-workers (2016), revealed that microemulgel prepared by screening of oils, emulsifier and co-emulsifier are more soluble as compared to gel and other topical preparations. These workers also suggested that microemulgel has an advantage over emulgel that they have dual benefits of microemulsion and gel and possess desirable properties such as good consistency, thyrotrophic, greaseless, easily spreadablility as well as removable from skin. Also these microemulgel are emollient, non-staining, water soluble and have longer shelf-life [6].

Gel formulations provide better application property and stability in comparison to cream and ointments. Skin is one of the most extensive and readily accessible organs of the body and best suitable for topical administration for local action or percutaneous penetration of medication for protective action. Recently besides emulgels, other preparations such as bigels and aerogels are also good for topical applications [19]. The gel formulations have been emerging as good topical drug delivery system due to their unique features. However, despite of providing many benefits, the gel faces several limitations in delivering hydrophobic drug molecules via skin. Therefore, in order to counter this problem, emulsion based formulations are being used for the delivery of hydrophobic drugs. Therefore, emulgel with dual drug release properties could be a better drug delivery option for topical applications [8].

3.0 Aim: Development of topical polyherbal emulgel and evaluation of its antimicrobial and anti-inflammatory activities.

The present study has been designed for the synthesis and characterization of polyherbal emulgel using plant sources for topical drug delivery and will be evaluated for its antimicrobial and anti-inflammatory studies. These formulations will have the natural drug components which will be safer for use and will have lesser associated side effects. The present study will be undertaken with the following objectives:

3.1 Objectives:

1. Preparation and evaluation of polyherbal emulgel formulation from marketed plant extract and essential oils.
2. *Ex vivo* evaluation of anti-inflammatory and antimicrobial activity of emulgel formulation.
3. To evaluate *in vivo* anti-inflammatory activity of herbal emulgel.

4.0 Materials and methods

4.1 Purchase of essential oils and test strains

Plant essential oils for the preparation of polyherbal emulgel, will be purchased from the local market. The following plant essential oils will be selected.

Sr. No.	Essential oil/Plant name
1	Tulsi (*Ocimum sanctum Linn*) [20]
2	Lemongrass (*Cymbopogon citrates*) [21]
3	Clove (*Syzygium aromaticum*) [22]
4	Coconut (*Cocos nucifera*) [23]
5	Eucalyptus (*Eucalyptus globules)* [24]

For the analysis of antimicrobial activity, the test bacterial and fungal strains will be obtained from the Biotechnology Department of the University (H.P).

4.2 Purchase of aloe vera plant extracts

The Aloe vera (*Aloe barbadensis*) will be purchased from……...

4.3 Screening of plant extract for preliminary phytochemicals

The plant extract (Aloe vera) will be screened for the detection of preliminary phytochemicals according to the procedure described by Salna et *al.,* 2011. The extract will be tested for the presence of different compounds such as alkaloids, tannins, saponins, quinone, coumarin, sugar and gum [28].

4.3.1. Detection of alkaloids

Detection of alkaloids will be done by Mayer's test. Test formulation will be dissolved in a few drops of 2N hydrochloric acid. The contents will be shaken and the aqueous layer will be decanted followed by addition of one or two drops of Mayer's reagent to the residue. Appearance of white turbidity or precipitate will indicate the presence of alkaloids.

4.3.2 Detection of tannins

Test material will be mixed with one or two drops of basic lead acetate solution. Formation of white precipitate will indicate the presence of tannins.

4.3.3 Detection of saponins

The test material will be mixed with a few drops of water and shaken well. Foamy lather formation will indicate the presence of saponins.

4.3.4 Detection of quinones

A few drops of sodium hydroxide will be added to the test material. Formation of blue green or red color will indicate the presence of quinones.

4.3.5 Detection of coumerin

To the test material, a few drops of 10% sodium hydroxide and chloroform will be added. Formation of yellow color will indicate the presence of coumerin.

4.3.6 Detection of sugar

The presence of sugars will be detected by Fehling's test. The test material will be mixed with equal volume of Fehling A and Fehling B solutions followed by heating in a water bath. Formation of red color will indicate the presence of sugar.

4.3.7 Detection of gum

One or two drops of water will be added to the test material and shaken well. Formation of swells or adhesiveness will indicate the presence of gum.

4.4 Preparation of polyherbal emulgel

The herbal based Emulgel will be prepared as per methodology described by Ashara and co-workers (2016). The oil phase (essential oils) will be added in aqueous phase (plant extract of aloe veera) and allowed to mix properly with a magnetic stirrer followed by addition of surface active agent (tween 80) for the proper dispersion of oil phase in aqueous phase. The emulsion phase thus prepared will be incorporated into a gel base by adding a gelling agent and a permeation enhancer [6].

4.5 Evaluation of emulgel

4.5.1 Viscosity

Cone and plate rotational viscometer with spindle will used to measure viscosity of the formulation.

4.5.2 pH

pH of the formulation will be measured using a digital pH meter.

4.5.3 Physical stability and appearance

The physical stability of the emulsion will be determined by centrifugation at ambient temperature at 5000 RPM for 10 minutes to evaluate the system for creaming or phase separation. The drug System will be observed visually for appearance [29].

4.5.4 Electric conductivity

The electrical conductivity of emulsion will be measured at ambient temperature with digital conductometer.

4.5.5. Spreadability

The spreadability of the emulgel will be measured by taking 0.5 gm of emulgel on a glass slide and a circle will be made around it. A second slide will be placed over it and a predetermined weight is put on it for specific time period. The increase in diameter will be noted as gm-cm/sec. [18].

4.5.6 Extrudability test

This test will be used to determine force necessary for removal of emulgel from tube and necessary to evaluate emulgel formulation for extrudability [30].

4.6 *In vitro* antimicrobial susceptibility of herbal emulgel against bacterial strains

The herbal emulgel formulation will be evaluated for its antimicrobial activity against bacterial strains using disc diffusion method.

4.6.1 Preparation of inoculum

Inoculums were prepared by suspending 5-8 colonies of each organism from the fresh cultures in 5ml of normal saline in a test tube followed by vortexing. The turbidity of each suspension was compared with 0.5 Macfarland standards.

4.6.2 Antibacterial assay

The disc diffusion method will be followed for determining inhibitory activity of the herbal emulgel against bacterial isolates using Muller Hinton Agar plates. The medium will be sterilized by autoclaving at 121°C for 15 minutes and poured in Petriplates. The sterilized cotton swabs dipped in bacterial inoculum will be swabbed over agar plates in order to spread the inoculum uniformly. Sterile discs containing 0.5mg of the herbal emulgel formulation will be placed on the surface of the inoculated medium and the plates will be incubated at 37°C for 24-48 hours. All the plates will be observed for the inhibition of the growth around the discs thereafter. The antibacterial activity will be determined by measuring the diameter of zones of inhibition of bacterial growth around the discs. The standard antibiotics discs will be used as positive control and discs dipped in DMSO as negative control for each test [31].

4.6.3 Evaluation of antifungal activity

Antifungal activity of the optimized gel formulation will be evaluated using agar well diffusion method as per methodology described by Pranali and co-workers (2019). The fungal suspension will be aseptically spread over sterile sabouraud's agar media plates. The surface of agar plates will be pierced by using a sterile cork borer. The prepared wells will be filled with an equal volume of emulgel formulation. The plates will be incubated at 18-24 ºC, for 72 hrs. The production of zones of inhibition around the well will be measured using antibiotic zone reader [32].

4.7 *In vitro* evaluation of anti-inflammatory effect

The *in vitro* anti-inflammatory studies will be done as per methodology described by Ranasinghe and co-workers (2012). Five ml of blood samples from a healthy volunteer and centrifuged it for 10 minutes at 3000 RPM. The blood cells will be washed with the normal saline thrice and the finally 10% v/v suspension of RBCs will be prepared adding normal saline. One ml of this mixture and one ml of test material (100 mcg/ml)

will be mixed and the mixture will be incubated in a water bath at 56 ºC for 30 minutes and then centrifuged at 2500 RPM for five minutes. The absorbance of the supernatants will be recorded at 560 nm. The aspirin will serve as positive and normal saline as negative control for the experiment. The results will be expressed as percent hemolysis as follow:

H (%) = {(Ac-As)/Ac} 100, where Ac and As are the absorbance of control and test samples respectively [33].

4.8 *In vivo* anti-inflammatory studies

The in vivo evaluation of anti-inflammatory activity of the prepared emulgel will be done as per methodology described by Mahajan and Bhaskar (2019) [34]. Edema will be induced on the left paw of the rats by subplantar injection of 1% (w/v) carrageenan. The emulgel formulation and standard diclofenac sodium gel will be applied 30 minute before carrageenan administration. The paw volume will be measured at interval of 30, 60, 90 and 120 minutes by mercury displacement method using plethysmometer. The experimental animals will be divided into three groups, Group-1 will be referred as control group carrageenan 1% will be administered at plantar surface, group-2 will be standard group, topical diclofenac gel and carrageenan will be administered and group-3 will be categorized as test group in which emulgel formulation and carrageenan will be administered. The % inhibition of paw edema in treated group will be compared with carrageenan control group and calculated as per formula: % inhibition of drug = (Vc-Vt/Vc x 100, where, Vc= inflammatory increase in paw volume control group, Vt= inflammatory increase in paw volume in drug + carrageenan treated animals.

5.0 Tentative Plan of Work

The ultimate aim of our study will be achieved using following plan of work steps.

1. For the base of emulgel, the aloe veera plants will be purchased from ……..while the essential oils of different plants i.e. tulsi (*Ocimum sanctum Linn),* lemongrass (*Cymbopogon citrates*), clove (Syzygium aromaticum), Coconut (*Cocos nucifera*) and Eucalyptus (*Eucalyptus globules)* will be purchased from the local market. For the analysis of antimicrobial activity, the test bacterial and fungal strains will be obtained from the Biotechnology Department of the University (H.P).

2. The Aloe vera extract/gel will be screened for the detection of preliminary phytochemicals such as alkaloids, tannins, saponins, quinone, coumarin, sugar and gum using standard protocol defined in section 4.3.

3. The ployherbal emulgel will be prepared by adding selected essential oils (oil phase) to the aloe vera extract/gel along with surface active agent (section 4.4)

4. The prepared polyherb emulgel will be optimized and evaluated for its viscosity, pH, stability, electric conductivity, spreadability and extrudability. (section 4.5).

5. The *In vitro* antimicrobial susceptibility of herbal emulgel against bacterial as well as fungal strains will be tested. The emulgel will be evaluated for its antibacterial potential against selected bacterial strains using disc diffusion method (Section 4.6.2). Similarly, the agar well diffusion method will be employed for the detection of antifungal potential of prepared emulgel against selected fungal strains (Section 4.6.3).

6. The polyherbal emulgel will be further evaluated i*n vitro* and in vivo for anti-inflammatory effect following standard protocol decribed in section 4.7 and 4.8 respectively.

7. All the results will be compiled and the best optimized polyherbal emulgel will be identified that may be further commercially developed.

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